Remarks

Claims 1, 11-13, 15, 17-23, 26-27 are pending. New Claims 30 and 31 have been added. Claims 1 and 11 have been amended. Support for the amendments to Claims 1 and 11 can be found in the previously presented Claims 1 and 11, respectively. Support for new Claims 30 and 31 can be found in Claims 1 and 22, respectively. No new matter has been added.

Based on the changes above and the following remarks, reconsideration of the claims is respectfully requested.

Response to Section 112, second paragraph

Claims 1, 11-13, 15, 17-21 and 26-27 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The rejection particularly points out that Claim 1 recites the limitation "the amino acid sequence," which supposedly lacks sufficient antecedent basis. Similarly, Claim 11 is rejected because it is not clear if the nucleic acid molecule is isolated and purified or only the nucleic acid sequence is isolated and purified. The Applicants note the Examiner's helpful suggestion with respect to amending Claim 11.

The Applicants have amended Claim 1 to recite that the isolated and purified protein comprises an amino acid sequence, thereby providing the appropriate antecedent basis. Claim 11 has also been amended in accordance with the Examiner's helpful suggestion. As a consequence, the Applicants respectfully submit that all of Claims 1, 11-13, 15, 17-21 and 26-27 are in full compliance with 35 U.S.C. §112, second paragraph. Withdrawal of the rejection is respectfully requested.

Response to Section 101 rejections

Claim 11 has been rejected under 35 U.S.C. §101 because the claim is directed to non-statutory subject matter. Amended Claim 11 is now directed to "an isolated and purified nucleic acid molecule comprising the nucleic acid sequence coding for the protein according to claim 1." The Applicants accordingly respectfully request that the portion of the rejection directed to Claim 11 be withdrawn.

Claims 1, 11-13, 15, 17-23, 26-27 remain rejected under 35 U.S.C. §101 because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility. More precisely, the rejection states that no disclosure is provided within the instant specification on what function the claimed cationic channel protein possess, nor any disease states disclosed that are directly related to claimed channel dysfunction.

The Applicants respectfully submit that the record supports well established utility. For **ASIC channels and ischemia**, this application clearly teaches the link between ASIC channels and ischemia to one skilled in the art in view of their general knowledge. This application discloses the fact that (i) The ASIC channels are homologues of the "degenerins" of the nematode C.elegans, and "Gain-of-function" mutation of the degenerins mec-4 and deg-1 are the principal known cause of neurodegeneration in the nematode (Chalfie, M. and Wolinsky, E. (1990). The identification and suppression of inherited neurodegeneration in Caenorhabditis elegans. *Nature*, **345**, 410-416. Driscoll, M. and Chalfie, M. (1991). The Mec-4 gene is a member of a family of *Caenorhabditis elegans* genes that can mutate to induce neuronal degeneration. *Nature*, **349**, 588-593. Huang, M. and Chalfie, M. (1994). Gene interactions affecting mechanosensory transduction in Caenorhabditis elegans. *Nature*, **367**, 467-470.).

The results described in this application and those neurodegeneration data, which were published before the filing date of this application, clearly teaches those skilled in the art that ASIC channels are implicated in neurodegeneration.

> *"It has been shown that the MDEG channel is activated by the same mutations as those causing neuronal degeneration in C. elegans. Thus, like the hyperactive degenerine mutants of C. elegans, the active mutants of MDEG are responsible for a cell death, indicating that the acquisition of function by this neuronal ionic channel could be implicated in various forms of neuronal degeneration of mammals, notably of humans. However, no normal physiological function of MDEG was known until the demonstration of its activation by protons in accordance with the cationic channels of the present invention." See Page 2, line 27.*

Moreover, it was also known in the art before the filing date of this application, that excessive Ca2+ entry plays an important role in neurodegeneration during ischemia (Choi, D.W. (1995) Calcium: still center-stage in hypoxic-ischemic neuronal death. *Trends Neurosci*, **18**, 58-60.).

Consequently, the fact that this application teaches that ASIC channels are expressed in brain (PA Fig.7) and that ASIC1 (SEQ ID 1) is permeable to Ca^{2+} (PA Fig. 5e) further demonstrates to one skilled in the art that ASIC channels are implicated in the neurodegeneration during ischemia.

Finally, it was also well known in the art that brain ischemia is associated with brain acidosis (pH < **6.5**) and that stronger acidosis aggravates brain injury (Siesjo, B.K., Katsura, K. and Kristian, T. (1996) Acidosis-related damage. *Adv Neurol*, **71**, 209-233; discussion 234-206). ASIC channels such as ASIC1 (SEQ ID 1) are already activated by slightly acidic pH (pH<6.9) and reach half maximal activity at pH 6.2 (PA Fig. 5b). Thus, brain pH during ischemia becomes sufficiently acidic to activate ASIC channels and confirm the potential implication of ASIC channel in neurodegeneration during ischemia.

In conclusion, this application teaches one skilled in the art a role of the ASIC channels in ischemic neuronal death and the utility of ASIC channel blockers as drugs that can be used to limit postischemic neuronal death.

The usefulness of ASIC channel blockers has been confirmed:

(i) a post-filing publication shows a role of ASIC channel activity in postischemic neurodegeneration and a neuroprotection by ASIC channel blockers (Xiong, Z.G., Zhu, X.M., Chu, X.P., Minami, M., Hey, J., Wei, W.L., MacDonald, J.F., Wemmie, J.A., Price, M.P., Welsh, M.J. and Simon, R.P. (2004) Neuroprotection in ischemia; blocking calcium-permeable Acid-sensing ion channels. *Cell*, **118**, 687-698).

(ii) A US patent application entitled "Inhibitors of proton-gated cation channels and their use in the treatment of ischaemic disorders" (US patent application **20060079529)** was filed by others.

Moreover, and for **ASIC channels and pain perception**, this application also clearly teaches the link between ASIC channels and pain perception to one skilled in the art in view of their general knowledge.

It was well known in the art that acids cause severe pain when applied to skin lesions or mucous membrane (Keele and Armstrong, 1964). Pronounced acidification accompanies inflammation or ischemia when the local pH drops to values as low as 5.4 (Häbler, 1929; Jacobus et al., 1977). Acidosis is also sufficient to provoke pain since intracutaneous injection of acid into human volunteers provoked pain (Steen, K.H. and Reeh, P.W. (1993). Sustained graded pain and hyperalgesia from harmless experimental tissue acidosis in human skin. *Neurosci Lett*, **154**, 113-116.). Thus, tissue acidosis was known to be a mediator of pain. The control of discharge and, thus, pain signaling of nociceptive ("pain sensing") neurons depends ultimately on the activity of membrane ion channels. Thus, ion channels in sensory neurons that are directly activated by pain mediators such as extracellular acid are evidently highly interesting targets for the development of analgesic drugs. A Na+ permeable, amiloride blockable ion channel that is activated by extracellular acid was described in 1981 in sensory neurons (Krishtal, O.A. and Pidoplichko, V.I. (1981). A receptor for protons in the membrane of sensory neurons may participate in nociception. *Neuroscience*, **6**, 2599-2601; ref. 3 in the application).

The link between acid-sensitive ion channels and pain perception is clearly mentioned in this application:

> *"Sensitivity to acid is associated with both nociception [I] and the transduction of taste 121. The stimulation of sensory neurons by acids is of great importance because acidity accompanies numerous painful inflammatory and ischemic situations. The pain caused by acids is interpreted as being mediated 15 by the cationic channels* present at the level of the sensory neurons and which are activated by protons [3-5]. The biophysical and pharmacological properties of the ASIC channels of the invention are close to those of the

proton-activated cationic channels described in the sensory neurons [3, 15, 16]." See Page 1, line 11 to 18.

The molecular structure of this acid sensing ion channel in pain sensing sensory neurons remained unknown until the Applicants cloned and functionally expressed the ASIC channels. Initially it was suggested that the acid sensing ion channel described by Krishtal et al. was a voltage gated calcium channel that changes ionic selectivity at acidic pH (Davies, N.W., Lux, H.D. and Morad, M. (1988). Site and mechanism of activation of proton-induced sodium current in chick dorsal root ganglion neurones. *J Physiol*, **400**, 159-187). Thus, the molecular structure of this acid sensing cation channel could not readily be deduced from its properties with the cloning of the ASIC channels. The Applicants defined the molecular structure of this acid sensing ion channel in sensory neurons and defined a novel highly interesting target for the development of analgesic drugs.

The role of ASIC channels in pain perception and, thus, the utility of the rejected claims was evident to one skilled in the art in view of their general knowledge and of the results of this application. It should also be noted that several groups started intensive research on the role of ASIC channels in pain perception after the Applicants published the data presented in this application.

ASIC3 channel activity was shown to be effectively responsible for mechanical hyperalgesia (Sluka, K.A., Price, M.P., Breese, N.M., Stucky, C.L., Wemmie, J.A. and Welsh, M.J. (2003). Chronic hyperalgesia induced by repeated acid injections in muscle is abolished by the loss of ASIC3, but not ASIC1. *Pain*, **106**, 229-239) induced after muscular acidosis – hyperalgesia was absent in ASIC3 null mice. Thus, this result confirms that blockers of ASIC3 should be efficient for the treatment of pain such as hyperalgesia associated with a tissue acidosis (e.g. during inflammation). Cardiac ischemia was also shown to be effectively associated with acidosis and pain, and the principal acid sensor in cardiac sensory neurons is ASIC3 (Benson, C.J., Eckert, S.P. and McCleskey, E.W. (1999) Acid-evoked currents in cardiac sensory neurons: A possible mediator of myocardial ischemic sensation. *Circ Res*, **84**, 921-928; Immke, D.C. and McCleskey, E.W. (2001) ASIC3: a lactic acid sensor for cardiac pain. *ScientificWorldJournal*, **1**,

510-512; Immke, D.C. and McCleskey, E.W. (2003) Protons open acid-sensing ion channels by catalyzing relief of Ca2+ blockade. *Neuron*, **37**, 75-84; Sutherland, S.P., Benson, C.J., Adelman, J.P. and McCleskey, E.W. (2001) Acid-sensing ion channel 3 matches the acid-gated current in cardiac ischemia-sensing neurons. *Proc Natl Acad Sci U S A*, **98**, 711-716). Thus, blocking of ASIC3 should be a means to relief angina pain.

All those publications, with nearly 250 others mostly dealing with pain perception and neurodegeneration, cited the article corresponding to this application (Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C. and Lazdunski, M. (1997) A proton-gated cation channel involved in acid-sensing. *Nature*, **386**, 173-177). were initiated by the data presented in this application, and confirmed the link between ASIC channels and ischemia, and between ASIC channels and pain perception established by the teaching of this application.

Finally, this application clearly teaches one skilled in the art specific functions as well as disease states related to the channel. Consequently, the claimed subject matter is fully supported by a specific and substantial asserted utility. The Applicants respectfully request withdrawal of the 35 U.S.C. §101 rejection.

Response to Section 112 (New matter) rejection

Claim 1 has been rejected under 35 U.S.C. §112 as failing to comply with the written description requirement. More precisely, the rejection states that no support can be found for the recitation "part or all" of a mammalian neuronal cationic ASIC channel selected from the group consisting of SEQ ID NO:2, 4 or 8. Amended Claim 1 is now directed to "an isolated and purified protein constituting a mammalian neuronal cationic ASIC channel that is sensitive to amiloride and activated by protons, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 8". "Part or all" has therefore been cancelled. That renders the rejection moot. The Applicants respectfully request withdrawal of the 35 U.S.C. 112 (New matter) rejection.

Response to Section 112, 1st paragraph rejection

Claims 1, 11-13, 15, 17-23, 26-27 remain rejected because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility.

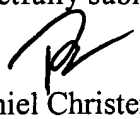
Claims 1, 11-13, 15, 17-23, 26-27 remain rejected as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. More precisely, the rejection states that the isolated and purified protein constituting **part or all** of a mammalian neuronal cationic ASIC channel.

Amended claim 1 is now directed to "an isolated and purified protein constituting a mammalian neuronal cationic ASIC channel that is sensitive to amiloride and activated by protons, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, and SEQ ID NO: 8".

The Applicants respectfully request withdrawal of the 35 U.S.C. 112, 1st paragraph rejection.

Based on the foregoing, the Applicants respectfully submit that the Application is now in a condition for allowance, which is respectfully requested.

Respectfully submitted,



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